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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:		
ROBERTSON ET AL.	Art Unit:	1642
Serial No. 09/857,739	Examiner:	Yu, M.
Filed: June 8, 2001		
For CANCER DETECTION METHODS AND REAGENTS		

DECLARATION UNDER 37 C.F.R. § 1.132 BY JOHN ROBERTSON

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

I, John Robertson, declare that:

1. I am a co-inventor of the above-identified patent application.

2. I received an MB ChB (equivalent of M.D. in US) from the University of Glasgow in 1980; an M.D (post-graduate Doctorate in Medicine) from the University of Glasgow in 1989; and am currently an Oncology Surgeon, a Principal Scientist for three research laboratories, and a Professor of Surgery, all at the University of Nottingham, Nottingham, England. I have over 200 peer reviewed publications and have been the editor of numerous book chapters and two books, all focused in the areas of cancer, blood markers, endocrine therapies and prognostic factors. I have been an invited speaker at numerous international conferences on the topic of blood markers in cancer.

3. The following experiments were conducted at my instructions and under my supervision to demonstrate the specificity of autoantibodies for cancer-associated tumor marker proteins. These highly specific autoantibodies are useful in assays to provide extremely sensitive methods for the detection of cancer-associated marker proteins in

patient samples, thereby facilitating early diagnosis and treatment to promote cancer patient survival.

Human autoantibodies to the tumor marker protein MUC1 were isolated by immuno-affinity chromatography from seroma fluid collected from a patient diagnosed with soft tissue sarcoma. The purified autoantibodies were then immobilized on a microtiter plate and assessed for reactivity with various MUC1 tumor marker proteins or the tumor marker protein MUC2.

As shown in attached Figure 1, purified autoantibodies (obtained by immuno-affinity chromatography with the patient's own sarcoma-derived MUC1) were immobilized and assessed for reactivity with the following:

1) normal MUC1 from the urine of a normal individual ("NED");
2) cancer-associated MUC1 from the urine of the cancer patient two years before he was diagnosed with sarcoma ("2yrs Pre-cancer");
3) cancer-associated MUC1 from seroma fluid of the cancer patient after he was diagnosed with sarcoma ("Diagnosed Cancer"); and
4) a synthetic MUC1 peptide conjugated to BSA ("BSA-AG").

As demonstrated in the data set forth in Figure 1, **the autoantibodies were found to be more highly reactive with cancer-associated MUC1** than with either normal MUC1 or synthetic MUC1, thereby showing the specificity of autoantibodies to cancer-associated tumor marker proteins.

As shown in attached Figure 2, autoantibodies purified by immuno-affinity chromatography with three different MUC1 sources were compared with an anti-MUC1 monoclonal antibody for specificity to cancer-associated MUC1. The four antibodies were as follows: a) a mouse monoclonal antibody that recognizes an amino acid sequence of the core VNTR peptide ("C595"); b) autoantibodies from the cancer patient (described above) purified with the patient's own sarcoma-derived MUC1 ("MRP MRP abnormal MUC1 purified autoantibodies") c) autoantibodies from the cancer patient purified against advanced breast cancer-derived MUC1 ("MRP ABC purified"); and d) autoantibodies from the cancer patient purified against a 60mer peptide of MUC1 ("MRP

60mer purified autoantibodies"). These four antibody sources were then immobilized on microtiter plates and reacted with the following four different types of MUC1 antigen and with MUC2 as shown on the y-axis of Figure 2:

1) a synthetic MUC1 peptide (a single VTNR amino acid sequence) conjugated to BSA ("BSA-AG");
2) a larger synthetic MUC1 peptide (25 VTNR amino acid sequences) bound to BSA ("BSA-TAP");
3) the sarcoma patient's own cancer-derived MUC1 ("MRP abnormal");
4) MUC1 from the urine of a normal individual ("Normal MUC1"); and
5) MUC2 polypeptide ("MUC2").

Figure 2 shows that **all three autoantibodies recognize cancer-associated MUC1 ("MRP abnormal") significantly better than the monoclonal antibody (C595). In addition, the data demonstrated that all autoantibodies are highly specific for the cancer-associated MUC1 protein and have little or no immunoreactivity with normal MUC1, synthetic MUC1 peptides or MUC2.** In contrast, the mouse monoclonal antibody (C595) detected normal MUC1 and synthetic MUC1 peptides better than it did cancer-derived MUC1.

In order to demonstrate that other autoantibodies have specificities for their respective cancer-associated tumor markers, samples from patients diagnosed with a variety of cancers were analyzed as follows: Cancer-associated marker proteins were purified either i) from the serum of patients diagnosed with cancer or ii) by recombinant technology. The various cancer-associated marker protein preparations were plated out in microtiter plates and dried. Biological samples from patients diagnosed with cancer were incubated in the plates to allow binding between the tumor markers immobilized on the plates and autoantibodies specific for those tumor markers present in the patient samples. Labeled anti-human or secondary anti-mouse antibodies were added to each well for detection of the autoantibody-tumor marker complexes.

The results, shown in attached Figures 3-9, demonstrate that a wide variety of human autoantibodies are highly specific for their respective cancer-associated tumor marker proteins as follows:

Figures 3a and 3b show data for MUC1 and c-myc. In Figure 3a, patients with primary breast cancer (PBC) or advanced breast cancer (ABC) were shown to have autoantibodies specific for **MUC1** purified from the sera of breast cancer patients (see scatter plot values above delineation of background noise). These autoantibodies are not present in the control groups (i.e., normal population, patients with histological diagnosis of benign breast disease, or benign autoimmune disorders – see scatter plot values below background noise line). Likewise, Figure 3b shows that patients with primary breast cancer (PBC) or advanced breast cancer (ABC) have autoantibodies specific for **c-myc**; Figure 4a shows that patients with primary breast cancer (PBC) or advanced breast cancer (ABC) have autoantibodies specific for **p53**; Figure 4b shows that patients with primary breast cancer (PBC) or advanced breast cancer (ABC) have autoantibodies specific for **c-erbB2**; Figure 5 shows that patients with primary breast cancer (PBC) have autoantibodies specific for **BRCA1** (anti-BRCA1 autoantibodies were identified in an even higher percentage of breast cancer patients having a mutation in the BRCA1 gene); and Figure 6 shows that patients with primary breast cancer (PBC) have autoantibodies specific for **BRCA2**.

Similarly, Figure 7 shows that patients with colorectal cancer have autoantibodies specific for **ras** (when compared to normal controls or patients with colonic polyps). Interestingly, the percentage of colorectal patients with autoantibodies to **ras** is highest in early stage cancer (A)[1], demonstrating the value of this autoantibody in the method described in the present application as an early diagnostic tool. Figure 8 shows that patients with colorectal cancer have autoantibodies specific for **APC** (when compared to normal controls or patients with colonic polyps); and Figure 9 shows that patients with prostate cancer have autoantibodies specific for **PSA** when compared to normal controls.

[1] The letters A, B and C/D refer to Duke's staging, which is a method of assessing whether colorectal cancer is early or advanced. Duke's A is very early colorectal cancer. Duke's B is early colorectal cancer.

In summary, based on my knowledge and expertise, the data presented herein demonstrates that the autoantibodies, as described in the present application, are present in the blood of cancer patients compared to normal and control populations. Further data are also presented which demonstrate that autoantibodies are more specific than monoclonal antibodies and are able to distinguish between normal and pathological isoforms of a protein. In addition, data presented shows that the autoantibodies display a higher affinity for cancer-associated protein, but little or no affinity for normal protein or synthetic peptides of the protein. Furthermore, data shows that the autoantibodies outperform monoclonal antibodies because of their improved binding characteristics and provide a highly sensitive method for the detection of cancer-associated marker proteins, thereby facilitating early diagnosis of cancer to allow earlier treatment with the ultimate goal of enhancing cancer patient survival.

4. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issuing thereon.

25/11/04
Date

John Robertson, MD, MB, ChB

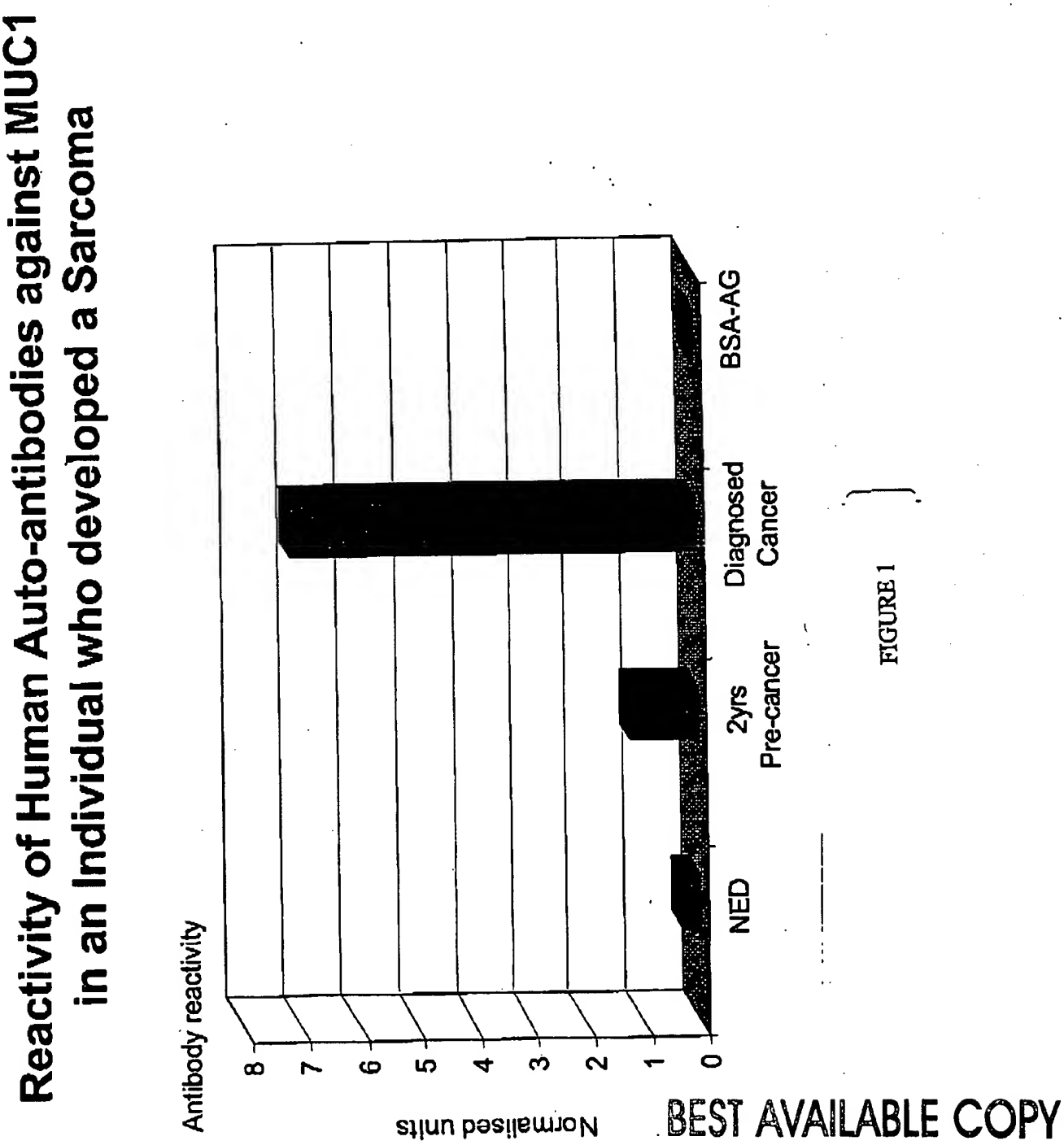

FIGURE 1

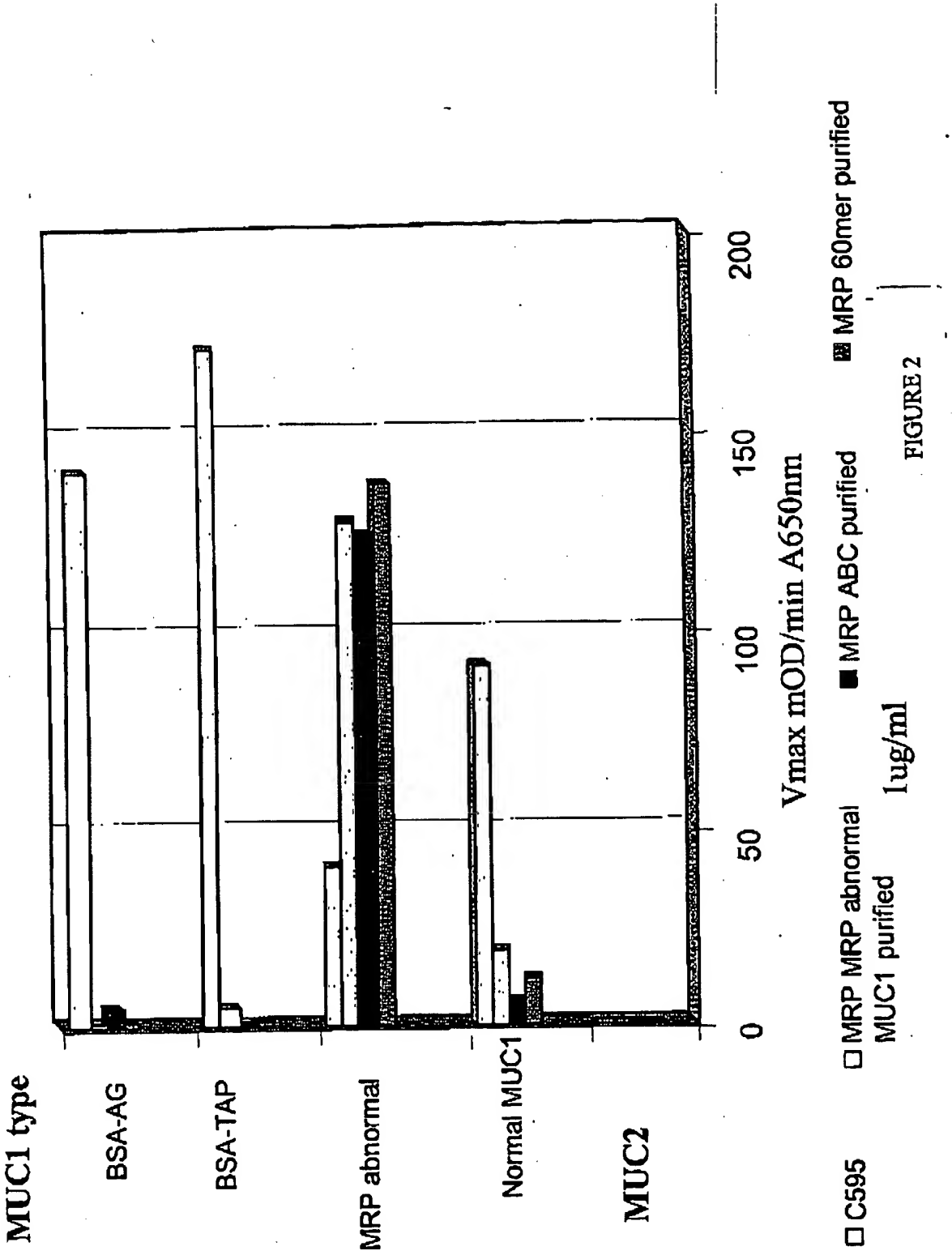
Seroma anti-MUC1 antibodies vs MUC1 types. (BSA blocked)
MUC1 type
BSA-AG
BSA-TAP
MRP abnormal
Normal MUC1
MUC2
0
50
100
150
200
Vmax mOD/min A650nm
C595
MRP MRP abnormal MUC1 purified
MRP ABC purified
MRP 60mer purified
1ug/ml

FIGURE 2

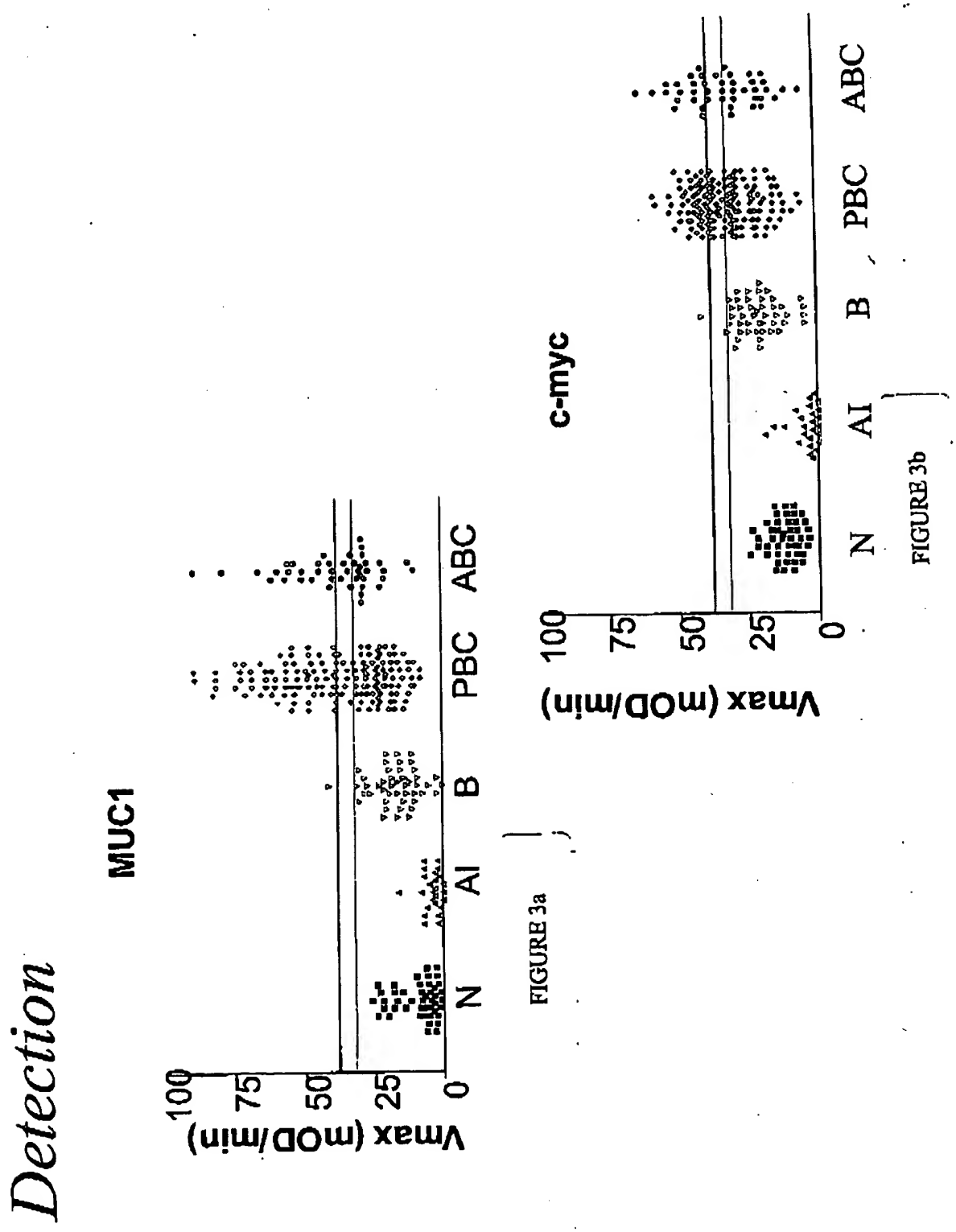

FIGURE 3a

FIGURE 3b

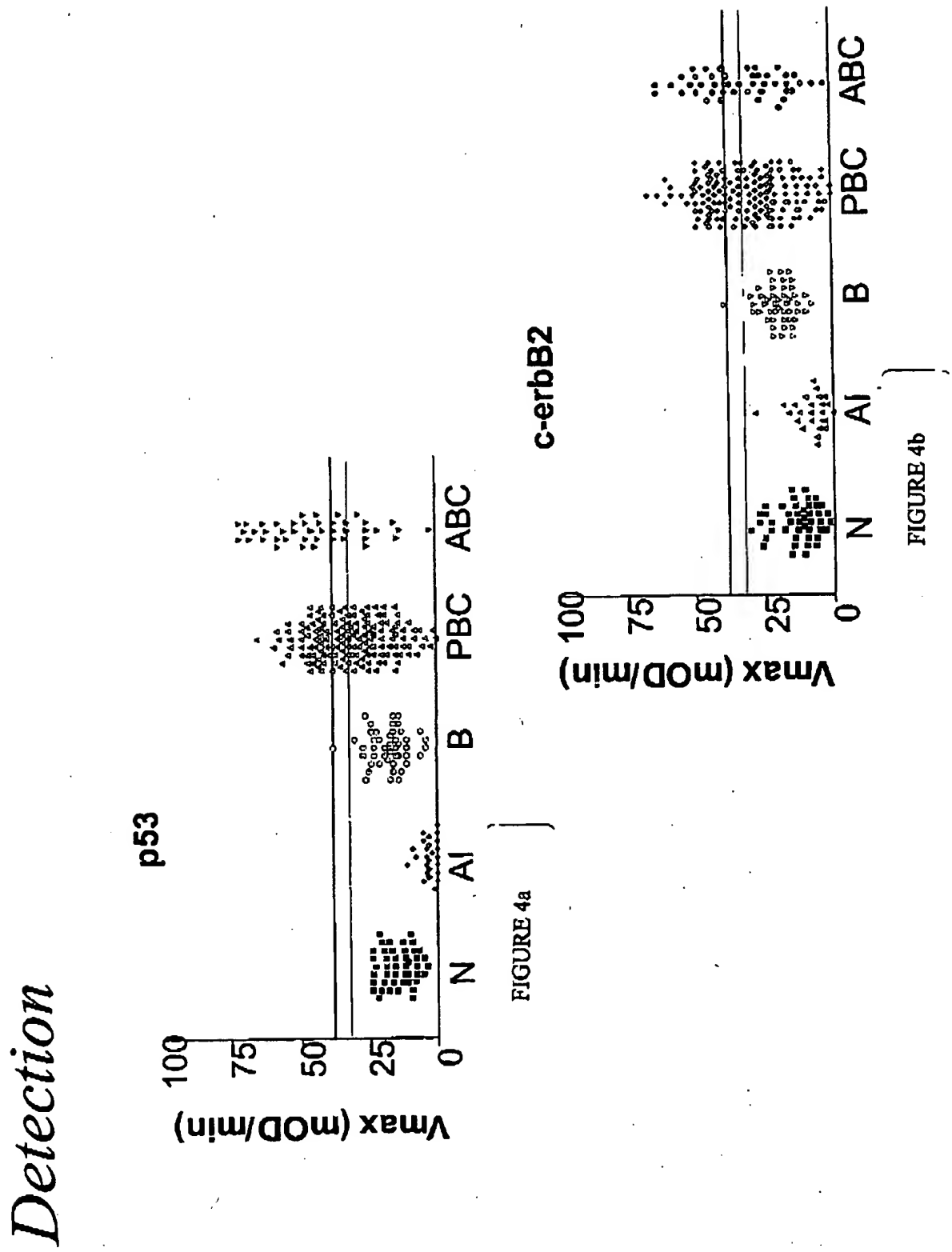

FIGURE 4a

FIGURE 4b

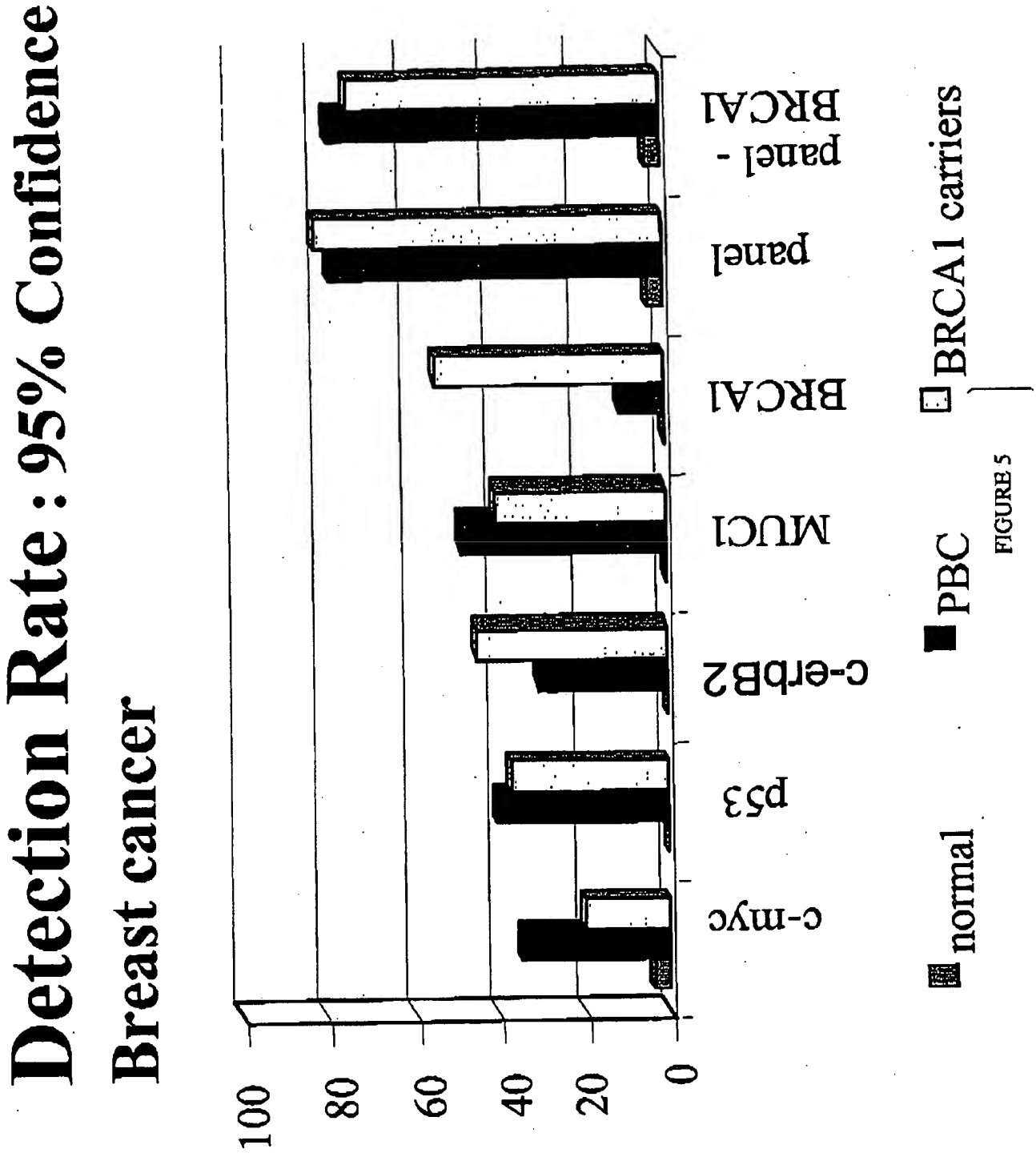
Detection Rate : 95% Confidence
Breast cancer
100
80
60
40
20
0
c-myc
p53
c-erbB2
MUC1
BRCA1
panel
panel - BRCA1
normal
PBC
BRCA1 carriers

FIGURE 5

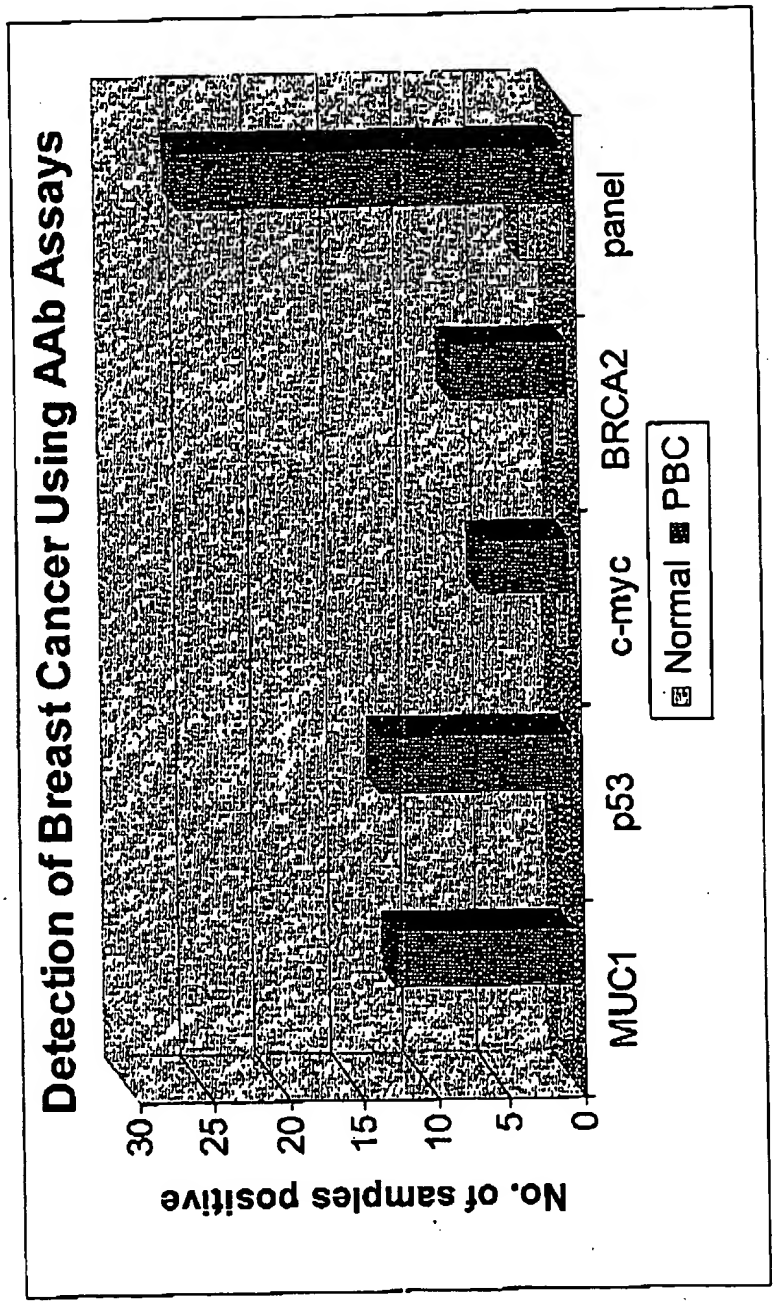
Detection of Breast Cancer Using AAb Assays
No. of samples positive
0
5
10
15
20
25
30
MUC1
p53
c-myc
BRCA2
panel
Normal
PBC

FIGURE 6

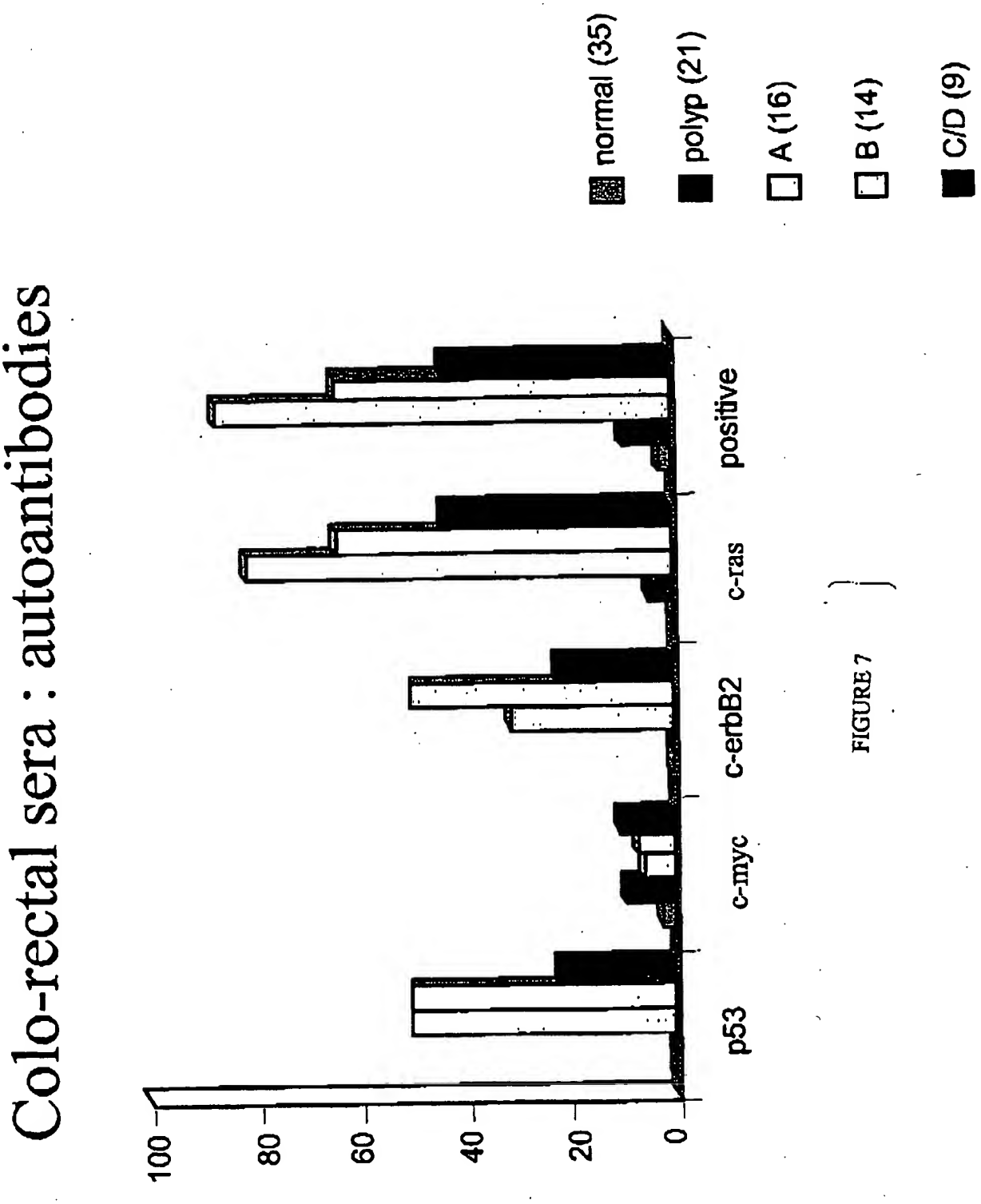
Colo-rectal sera : autoantibodies
normal (35)
polyp (21)
A (16)
B (14)
C/D (9)
100
80
60
40
20
0
p53
c-myc
c-erbB2
c-ras
positive

FIGURE 7

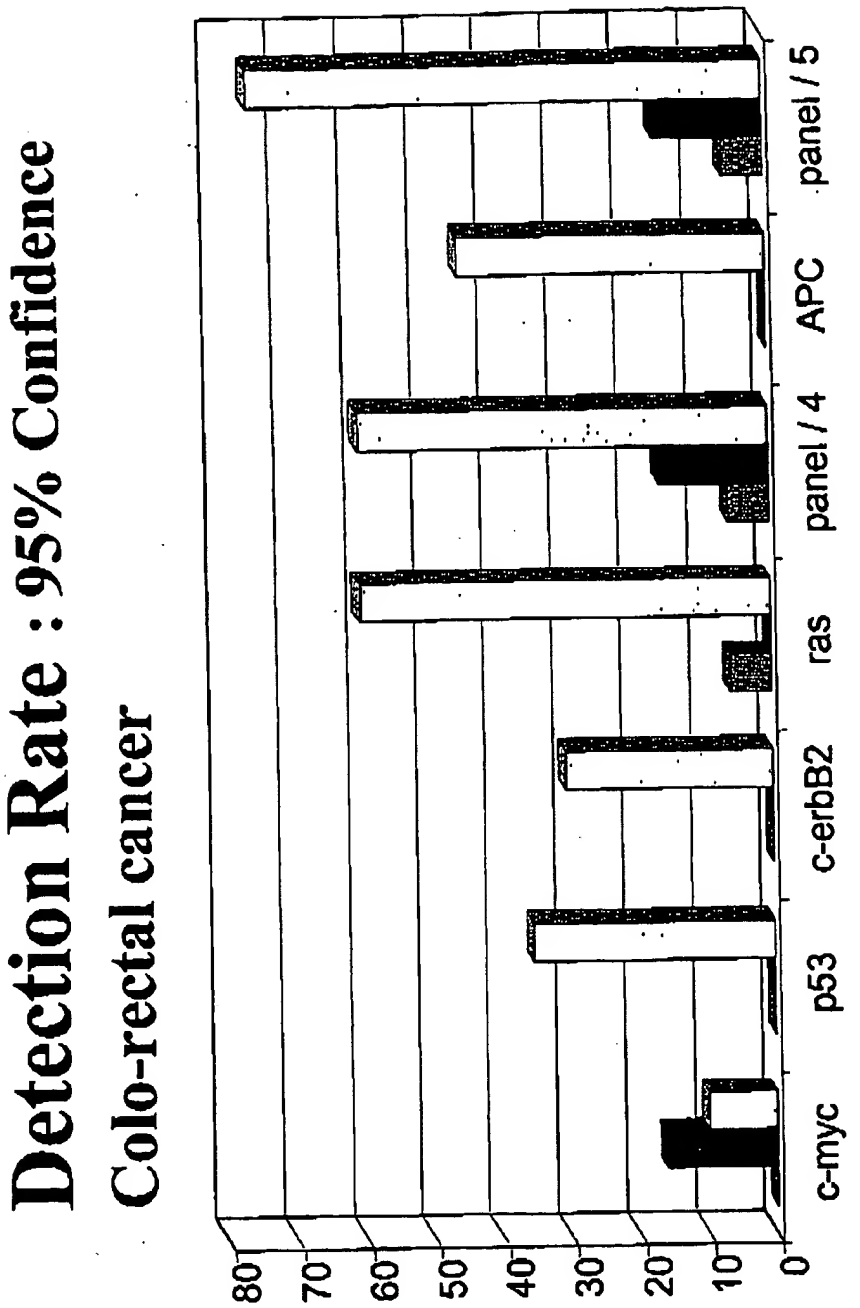
Detection Rate : 95% Confidence
Colo-rectal cancer
80
70
60
50
40
30
20
10
0
c-myc
p53
c-erbB2
ras
panel / 4
APC
panel / 5
normals
Polyps
C/R Cancer

FIGURE 8

FIGURE 9


120
80
60
40
20
0
c-myc
PAI
c-erbB2
NMC1
PSA
panel
normal
Prostate cancer